

HAEMOPHILUS

FOR IN VITRO DIAGOSTIC USE

INTENDED USE

PRO-LAB Haemophilus Selective Supplement is an antibiotic supplement used to enhance the isolation of Haemophilus sp.

SUMMARY AND EXPLANATION

Haemophili are non-motile, Gram-negative organisms which are strictly parasitic and, when being cultured in vitro, require growth factors present in blood, expecially X (Hemin) and/or V(NAD) factor. In culturing Haemophilus sp., difficulties are often encountered due to the overgrowth by more strongly growing commensal organisms.

Two groups Crawford, Barden and Kirkman¹ and Klein and Blazevic² reported the development of a medium for the selective isolation of *Haemophilus sp.* using bacitracin as a selective agent. It supported the growth of Haemophilus sp. while suppressing growth of all Grampositive bacteria and Neisseria sp. More recent work by Tempro and Slots³, confirmed the addition of bacitracin (75 mg/l) to the culture medium inhibits the growth of most commensal organisms resulting in a high recovery rate of Haemophilus sp.

DESCRIPTION

An accurate quantity of Bacitracin is lyophilized and provided in individually labelled vials, each vial being sufficient to supplement 1000 mls of prepared medium.

FORMULA

Each vial contains:

Bacitracin 75 mg

PROCEDURE

To reconstitute each vial of PRO-LAB Haemophilus Selective Supplement, add, aseptically, 10 ml of sterile distilled water. After reclosing the vial, gently agitate to assist reconstitution.

Prepare Columbia Agar according to the manufacturer's instructions, autoclave and cool to 55°C. Add 5-7% sterile, defibrinated horse blood, and mix thoroughly. Heat to 80°C, mixing occasionally, until the medium becomes a chocolate brown colour. Cool medium to 50°C. Add the reconstituted contents of one vial of PRO-LAB Haemophilus Selective Supplement PL.430 to a final 1000 ml prepared medium. Mix gently and pour into sterile petri dishes. Overnight storage at 4°C is recommended to allow suitable equilibration. For extended storage at 4°C, eg. up to 7 days, plates should be contained in sealed plastic sleeves or similar packaging.

IN USE

- 1. Before using selective medium ensure that plates are
- 2. Inoculate test material onto surface of agar using sterile inoculating loop (available from PRO-LAB) or a sterile swab in such a manner as to encourage the growth of isolated colonies.
- 3. Incubate plates at 37°C in an atmosphere containing 10% CO₂.
- 4. Examine after incubation (18-24 hours).
- 5. Suspect colonies of *Haemophilus sp.* should be further tested for X and V factor requirement.

SAFETY PRECAUTIONS

- 1. PRO-LAB Haemophilus Selective Supplement PL.430 is offered only as an in vitro material and is in no way intended for a curative or prophylactic purpose.
- 2. During and after use, handle all materials in a manner conforming to Good Laboratory Practices and consider at all times that material under test should be regarded as a potential biohazard if mishandled.

PRESENTATION

PRO-LAB Haemophilus Selective Supplement PL.430 is supplied 10 vials per box (lyophilized).

STORAGE

PRO-LAB Haemophilus Selective Supplement PL.430 must be stored at 2°C to 8°C. Kept under these conditions it may be stored up to date of expiry shown on product label.

REFERENCES

- 1. Crawford, J.J., Barden, L., and Kirkman, J.B. 1969. Appl. Microbiol. 18: 646-649.
- 2. Klein, M., and Blazevic, D.J. 1970. Am. J. Med. Tech. 36: 97-106.
- 3. Tempro, P.J., and Slots, J. 1986. J. Clin. Microbiol. 23: 777-782.

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